SOLID STATE STABILITY OF DIGOXIN AS A FUNCTION OF TEMPERATURE AND HUMIDITY

By

Arthur H. Kibbe

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL
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Major Department: Pharmacy

Digoxin in tablet form was subjected to various conditions of temperature and humidity. The results were statistically analyzed using a 3x3x2 factorial design. A thin layer quantitative assay was developed for single tablet determinations after a gas chromatographic approach failed. The results indicated a strong correlation between temperature and rate of decay as well as humidity and rate of degradation. A high degree of tablet to tablet variation was noted in addition to a difference between manufacturers. Tablet hardness, weight, and disintegration time for both manufacturers was checked. A comparison of the results with solution kinetic theory was made and an explanation for the inconsistencies attempted.

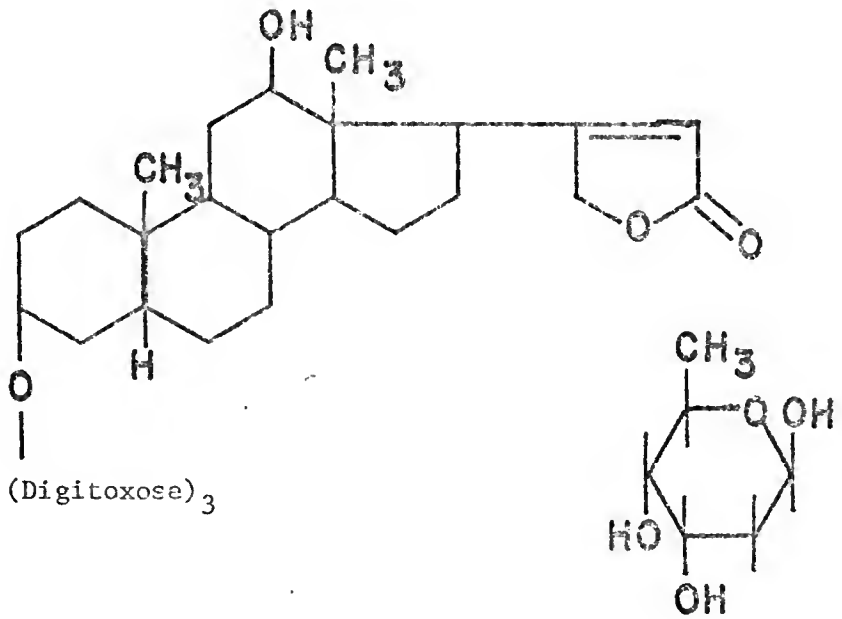
INTRODUCTION

Digoxin is a naturally occurring cardiac glycoside employed in medicine for the treatment of congestive heart failure. Cardiac glycosides, in one form or another, have been used since 1785 when William Withering published "An Account of the Foxglove and Some of its Medical Uses".

Digoxin was isolated from Digitalis lanata in 1930 by Smith.[1] Its plant precursor is lanatoside-C or digilamide-C and the digoxin molecule is formed by loss of a glucose and acetic acid molecule through hydrolysis.[2]

Digoxin is a colorless to white crystalline powder. It is insoluble in water, chloroform and ether. It is soluble in pyridine and dilute alcohol.[3]

The structure of digoxin consists of three components: (1) an α, β unsaturated lactone, (2) a steroid ring system and (3) three digitoxoses.



The U.S.P. XVIII monograph states: "Digoxin is a cardiotonic glycoside obtained from the leaves of Digitalis lanata Ehrh. (Fam. Scrophulariaceae). It contains not less than 96% $C_{41}H_{64}O_{14}$ calculated on a dried basis. CAUTION- Handle digoxin with exceptional care, since it is highly potent."[4] The preceding information is about all that is learned from the official compendium on the chemistry, stability and pharmacology of one of the most commonly used drugs among people over fifty years of age.

Digoxin is particularly important because of its nonmetabolic pathway of elimination, slow excretion and good reabsorption from the alimentary tract. Most individuals under digoxin therapy use tablets manufactured and marketed by one of several drug companies. The patients must maintain the necessary concentration of the drug in the cardiac tissue or relapse into congestive heart failure. It therefore becomes obvious that after two to three weeks of therapy with tablets below labeled potency, a dangerously low level of digoxin in the blood and heart can occur. These facts make the importance of an accurate daily dose quite apparent.

Most patients use a single tablet of digoxin daily as a means of obtaining their medication. The use of tablets as a dosage form can be traced back to the 16th century. Tablets can be made either by molding or compression. The United States Pharmacopeia defines a tablet as "a solid dosage form containing medicinal substances with or without suitable diluents." On a small scale, they may be prepared by various molding techniques or by fusion; however, large scale production requires the use of a tablet compressing machine. To efficiently and uniformly

operate such equipment a homogeneous mixture of solid particles possessing suitable physical characteristics is required.[5]

A well-made compressed tablet is able to withstand the stress involved in production, packaging, shipment and dispensing. When the patient receives the tablet, it should be free from obvious defects such as cracks, chipped edges, discoloration, specking and contamination. More importantly, however, it should be reasonably stable to chemical and physical change in the active ingredient, while maintaining its ability to release the medicament in a reproducible and predictable manner. All of this should lead to a consistent bioavailability from tablet to tablet.

Machines built to compress tablets are of two general types: the single punch and the multistation rotary press. Each performs the same task of converting the mixture (called a granulation) of drug, diluents, binders, lubricants and disintegrants into a tablet. The mixture must possess the essential characteristics of fluidity and compressibility. Fluidity is necessary because of the nature of the tabletting machines which requires the sized granules to flow from a storage hopper through a feed frame into the dye that shapes the tablet. If the granulation does not flow smoothly, vibrations may introduce a serious problem of stratification or separation of different size particles. This may cause changes in total tablet weight or uniformity of content or both. The ideal shape of the granulation particles, therefore, would be spheres since they would flow smoothly. Granulation is the pharmaceutical process that attempts to connect powdered materials into aggregates called granules that are approximate spheres.

Compressibility is the property of forming a stable, compact mass when pressure is applied. The bonding of particles in a tablet is probably due to a number of mechanisms. During compression, surfaces are brought into close proximity by plastic deformation. Mechanical interlocking, as well as electostatic and Van der Waals forces, combine to add to the strength of the compressed tablet.

Since granulation increases both fluidity and compressibility, almost all compressed tablets are formed from granulation produced by either the dry or wet method.

Diluents are employed as a bulking agent in tablets. Lactose, which is frequently used, is relatively inexpensive and is available in a coarse or regular granular size. Starch is also used, as well as the less common diluents mannitol, microcrystalline cellulose and sucrose.

Binders are substances that cause powders to adhere and form granules. They can be added dry and then activated with water or added as a slurry to the other powders. Acacia is a common binder used as a 10 to 20 per cent solution. Other binders that are used in various per cent solutions of water or water-alcohol are tragacanth, gelatin, sucrose, methylcellulose and polyvinylpyrrolidone.

Once the granulation is made, an additional additive, a lubricant, is used to improve flow properties and to prevent the tablet from sticking to the dyes and punches employed in the tabletting process. This is employed in a fine particle size and can be either a metallic stearate, high melting point wax or talc.

The last additive, the disintegrant, is one that will cause the tablet to break apart when placed in an aqueous environment. Most compounds used for this purpose swell when placed in water. Starch does

not, however, work in this manner since it does not swell at normal temperatures of gastric fluid. Curlin[6] suggests that it acts as a wick bringing aqueous fluid into the tablet where it dissolves the binding agent and causes other additives to swell. In addition to starch, which is the most common disintegrant, gums, cellulose derivatives and alginates may be used.

After granulating and tabletting, the final product is evaluated through several standard tests. These tests are the basis for guaranteeing quality control on a batch to batch basis. Some of these tests are hardness, disintegration time, weight and dissolution rate.

Traditionally, to determine tablet strength or hardness the tablet was broken between the thumb and first and second finger. If there was a sharp snap the tablet was satisfactory. Several companies have since developed hardness testers, all of which actually measure resistance to crushing. There is, however, no correlation between the scales of each tester.

A commonly used apparatus for determining tablet hardness is a Monsanto tester which consists of a barrel containing a compressible spring held between two plungers. As the spring is compressed by turning the threaded bolt, a pointer rides along a gauge in the barrel and indicates the pressure at which the tablet fractures.

The U.S.P. tablet disintegration test is specific for the type of tablet being tested. It consists of an apparatus that moves the tablet up and down through a test fluid maintained at a temperature between 35° and 39° at a rate of 28 to 32 cycles per minute with a stroke distance of from 5 to 6 cm. per cycle. The in vitro procedure does not actually simulate physiologic conditions. It is used, rather, as a means of

quality control to insure product uniformity. The test does not indicate complete solution of the tablet or even dissolution of its active constituents.

Wagner[7], Wurster and Taylor[8], and Pernarowski, Woo and Searl[9] all have worked on apparatuses for dissolution rate studies. The U.S.P. has since adopted an official device for this purpose. The tablet is placed in a test medium which is sampled at various time intervals and thus the rate at which the active ingredient goes into solution is determined. The test medium approximates the gastrointestinal fluid and the dissolution medium is maintained at 37° so as to closely parallel the in vivo process.

The last several revisions of the U.S.P. have included a requirement for weight variation tolerance. These requirements are generous in that they allow a 10 per cent difference in two tablets out of 20, for tablets weighing 130 mg. or less. Until recently, the assay of drug content of tablets involved the grinding of a large sample followed by analysis of an aliquot. Results obtained were then expressed on an individual tablet basis. Efforts to overcome this problem brought the content uniformity test described in the U.S.P.:

> Where a requirement of Content uniformity is specified in the individual monograph, select a representative sample of 30 tablets. Assay 10 of these individually as directed in the Assay in the monograph. If the amount of drug in a single tablet is less than that required in the assay procedure, the degree of dilution of the solutions and/or the volume of aliquots may be adjusted so that the concentration of the drug in the final solution will be of the same order as that obtained in the Assay provided in the monograph. The requirements of this test are met if all 10 results fall within the limits of 85 per cent and 115 per cent of the average of the tolerances specified in the respective monograph. If 1, but not more than 1.

> result falls outside these limits, assay the remaining 20 tablets individually. The requirements are met if not more than 1 of the 30 results is outside the limits of 85 per cent and 115 per cent.

From the preceding information, one can see that the formulation and manufacture of tablets is a complex process and thus is liable for some serious errors. This is especially true for tablets that contain a low percentage of active ingredient, such as digoxin tablets. A small change in the overall process might affect the properties of the active ingredient or its distribution between the tablets to an inordinate degree.

Various colorimetric assays for digoxin have been reported. Alkaline picrate was used by Baljet[10], xanthydrol by Pesez[11], and 3,5 dinitrobenzoic acid by Tattje.[12]

An automated assay of single tablets of digoxin which depend on chemical reactions such as the oxidation of the terminal sugar of the glycoside into a malonic dialdehyde was proposed by both Khoury[13] and Myrick.[14] A fluorometric micromethod for simultaneous determination of digitoxin and digoxin was proposed by Jakovljevic[15] while an automated fluorometric procedure was proposed by Cullen, Packman and Papariello.[16] These methods involve reactions, which can also occur with other glycosides and mono- or di-digitoxose aglycons and therefore render the assays non-specific.

Thin layer chromatography combined with colorimetric reactions, as well as gas chromatographic assay procedures, have been reported. They are all based on the theories of chromatography on thin layers of adsorbent which were conceived as early as 1938 and developed largely

during the early 1950's. Chromatography was developed because of a specific need for a rapid method of separating minute amounts of compounds. It was not limited to colored compounds because of the possibility of carrying out certain reactions which would make otherwise colorless compounds visible in either normal or ultraviolet light.

Chromatography can be considered from three viewpoints. One is qualitative, the second is preparative and the third is quantitative. All of the techniques are based upon a single simple principle involving a moving system of some type of gas or liquid in equilibrium with a stationary phase. When a solid is used as the stationary phase and the substances being separated are adsorbed onto it, the method is called adsorption chromatography. If, however, the stationary phase is a liquid or gas held on some type of support, it is partition chromatography.

Gas chromatography can be of either type. The advantages of gas chromatography are its speed of operation, its high degree of resolution and the fact that it can yield quantitative results. Its major disadvantage, other than high cost, is that the substances to be separated must have at least some vapor pressure at a workable temperature.

An ideal method of chromatography should lend itself to quantitative interpretation and should be technically simple in design. Both of these conditions are fulfilled by thin layer chromatography. The development time is shorter than that for paper chromatography and a number of procedures have been published for quantitative assay.

Quantitative evaluation of thin layer chromatograms fall into two general categories. In one case, a mixture is separated on the thin layer and then eluted for measurement by a spectrophotometric or colorimetric method. The second and simpler method involves the measurement of spot

area and these values are then related to substance amount in some manner.

The spot area analysis is based on a mathematical relationship between the spot area and the weight of a given substance. The method is widely applicable and involves only a small number of mechanical operations.[17-23] Seher obtained results with only 5 per cent error using calibration curves obtained by plotting spot area against weight.[24] Brenner and Niederwieser obtained a linear relationship using a plot of the logarithm of the weight of sample against its spot area.[25] The most complete study involving the technique was made by Purdy and Truter, who found a linear relationship using the square root of the spot area vs. the logarithm of its weight. They developed a simple algebraic relationship which for 540 observations yielded a standard deviation of 2.7 per cent.[26,27]

Eric Watson and S. Kalman assayed plasma levels of digoxin by gas chromatography. Their assay procedure took five hours per assay and involved both thin layer and gas chromatography steps.[28] All previous work on gas chromatography of digoxin had only been of a qualitative nature. Watson used electron capture detection while Wilson et al. used silyl ethers of cardenolides which required high temperature and high flow rates of the carrier gas.[29] A recent study by Tan questioned the formation of the silyl ethers reported by Wilson and others.[30]

Separation of digoxin from the other glycosides was achieved by Svendsen and Jensen.[31] Thin layer chromatography on digoxin was also employed by Stahl, who used methylene chloride-methanol-formamide as the solvent system.[32] Heusser proposed a method based on thin layer

chromatography separation of the glycoside and subsequent colorimetric determination using xanthydrol.[33]

Michalska, Zurkowaka and Ozarowski studied the stability of digitoxin at room temperature, 40°, and 47°. They reported, however, that their determinations of total glycosides do not correlate with the actual changes in therapeutic value of the drug. They further stated that the total glycoside content stayed constant over a period of seven months at elevated temperatures but that there was a decrease in digitoxin content. However, they omitted any discussion concerning the interconversions which would account for the decrease in digitoxin.[34,35]

Grollman states that all digitalis preparations are indicated for any of the forms of congestive heart failure irrespective of type of rhythm.[36] The action of the glycoside in treating atrial fibrillation is due to the specific effect of the strength of contraction and on conduction of the cardiac muscle. Digoxin has an onset of action of two hours if taken orally and of five minutes if given intravenously, with an average time of maximal effect at eight hours after oral and five hours after intravenous administration respectively. The duration of effect is from four to seven hours. The myocardium displays no special affinity for the cardiac glycosides which are present in highest concentration in the kidney. The glycosides are bound reversibly to the plasma albumin. A normally digitalized man excretes about 32 to 44 mcg. daily.[37]

Doherty, Hall, Murphy and Beard presented a critical review of digitalis metabolism. They reported that digoxin is 80 to 90 per cent absorbed, recycled to a small extent, only slightly protein-bound, poorly metabolized and excreted largely unchanged in the urine. Consequently, its half-life of 1.5 days is shorter than digitoxin and its excretion may be directly related to creatinine clearance.[38]

Cattel et al. state that digitoxin is 100 per cent absorbed.[39] The variation of this degree of absorption from the 80-90 per cent attributed to digoxin by Doherty can best be explained by the slight difference in structure of the two compounds, resulting in a change in polarity.[40] This difference was noted to have a large effect on the half-life since digitoxin is recycled and digoxin is not.[41]

Doherty and Perkins surgically induced biliary fistula in human subjects to prevent recycling. When digoxin was given orally to these subjects as well as to another group of normal humans, there appeared to be no difference in the serum half-life.[42]

Doherty recommended the following dosage regimen for digoxin: One to 2.5 mg. as a single dose orally in a 24-hour period with a maintenance dose of from 0.125 to 0.75 daily based on renal function, and organ response, absorption and excretion.[43]

The present study proposed to examine the effects of humidity and temperature on possible solid state degradation of different commercial tablets of digoxin, as well as to investigate tablet to tablet variation for digoxin. Where the U.S.P. assay calls for the use of 20 tablets to give an average content, individual tablet assay will be carried out in this study. In this manner an indication of the combined effect of these factors on tablet degradation can be ascertained.

EXPERIMENTAL

Digoxin tablets manufactured by two companies were used throughout this study: 1000 tablets (0.25 mg.) from Purepac Pharmaceutical Company, Division of Elizabeth Labs, Elizabeth, New Jersey, Lot #0192F3 and 1000 tablets (0.25 mg.) from Burroughs Wellcome Company, Research Triangle Park, North Carolina, Lot #196E. The tablets from each company were divided at random into groups of 100 and either used as zero time samples or placed in the containers for exposure to the various temperatures and relative humidities used in the degradation studies.

Desiccators containing sulfuric acid-water solutions provided the atmospheres for the three relative humidities employed, as shown in Table I.[44]

The desiccators for each relative humidity were stored at three different temperatures, 23°, 40° and 50°. The ovens used were Thelco, Model 6M, manufactured by Precision Scientific. The temperature was controlled to within one degree.

Assay Procedures

Gas-Liquid Chromatography

An attempt was made to quantify the work done by Wilson *et al.* using a Varian 2100 gas chromatograph equipped with a Hydrogen Flame Detector. The support was supplied by Applied Science complete with a precoating of 2.5 per cent OV-1 on Chromsorb W 80-100 mesh. The silanizing reagent was prepared just prior to use by mixing 1 ml. of trimethyl

chlorosilane and 10 ml. of hexamethyldisilazane with 10 ml. of dry pyridine as a solvent. The pyridine was stored over potassium hydroxide to insure dryness. The hexamethyldisilazane and trimethyl chlorosilane were stored under nitrogen and extracted from sealed vials with a syringe. The volume was then replaced with dry nitrogen gas. The sample to be silanized was made to react with 1 ml. of the above reagent at room temperature before being injected into the gas chromatograph.

Initially glass columns 6 feet in length with 2 mm. ID were used. Due to the high temperature of operation suggested by Wilson, difficulty was experienced in maintaining a firm seal between the glass column and the metal connections. The Teflon ferrel, which normally would be used for this purpose, began to melt at 300° to 350° temperature at which the injector and detector were operated. Red rubber "O" rings supplied by Applied Science were used to replace the Teflon ferrels. They, however, deteriorate rapidly at the high temperature.

To alleviate this problem the use of copper columns having a 4 mm. ID but only a 1 1/2 foot length was employed. This enabled the use of swage lock fittings which prevented leaking and slipping. Nitrogen was employed as a carrier gas in all the experiments and the rate of flow was varied from between 50 ml/min. to 100 ml/min.

The genins, as well as digoxin itself, were silanated separately and in mixture. Different concentrations of each were injected in volumes of between 2 to 5 microliters. After repeated injection over a period of days, a high noise level developed which required the column packing to be changed. This was very time consuming and introduced variations in the results since no two columns are exactly alike.

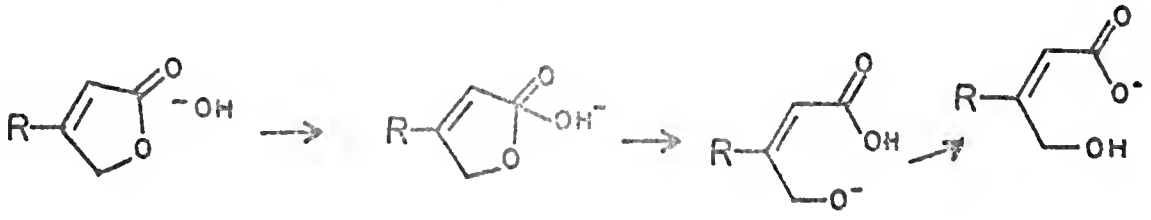
U.S.P. Assay

The U.S.P. assay was performed on groups of 20 tablets at the beginning and at the end of the stability studies.

> Alkaline dinitrobenzene solution---Mix 1 ml. of tetramethylammonium hydroxide T.S. with 140 ml. of dehydrated alcohol, titrate a portion of the solution with 0.01 N hydrochloric acid, and adjust the remaining solution to a concentration of 0.008 N by dilution with dehydrated alcohol. Immediately before use, mix 40 ml. of this solution with 60 ml. of a 1 in 20 solution on m-dinitrobenzene in benzene.
> Standard preparation---Dissolve 25.0 mg. of U.S.P. Digoxin Reference Standard, previously dried in vacuum at 105° for 1 hour, in 50 ml. of hot alcohol, cool, add alcohol to make 100.0 ml., and mix. Dilute 10.0 ml. of this solution with alcohol to 100.0 ml.
> Assay preparation---Weigh accurately about 25 mg. of Digoxin, and prepare solutions as directed under Standard preparation.
> Procedure--- Pipet 5 ml. each of the Assay preparation and the Standard preparation into separate, small conical flasks, and treat each solution as follows: Evaporate on a steam bath, with the aid of a current of air, to dryness, and cool the residue in a vacuum desiccator for 15 minutes. Add 5.0 ml. of Alkaline dinitrobenzene solution, and allow to stand, with frequent swirling, for 5 minutes at a temperature not exceeding 30°. Determine the absorbance of the solution, relative to a reagent blank, in a 1-cm. cell at 620 mμ with a suitable spectrophotometer, repeating the measurement at 1-minute intervals until maximum absorbance is obtained.
> Calculate the quantity, in mg., of $C_{41}H_{64}O_{14}$ in the portion of Digoxin taken by the formula $25(A_u/A_s)$, in which A_u and A_s are the maximum absorbances of the solutions from the Assay preparation and Standard preparation, respectively.

The absorbance was measured on a Fischer colorimeter. One possible mechanism which could lead to the development of the color in the U.S.P. assay is an electron transfer from the digoxin to the m-dinitrobenzene, which lowers its absorbance energy from 300 mμ to 620 mμ. In order for

digoxin to transfer this electron, a change in structure must be caused by the tetramethyammonium hydroxide. The probable steps in this reaction are shown in the following scheme.



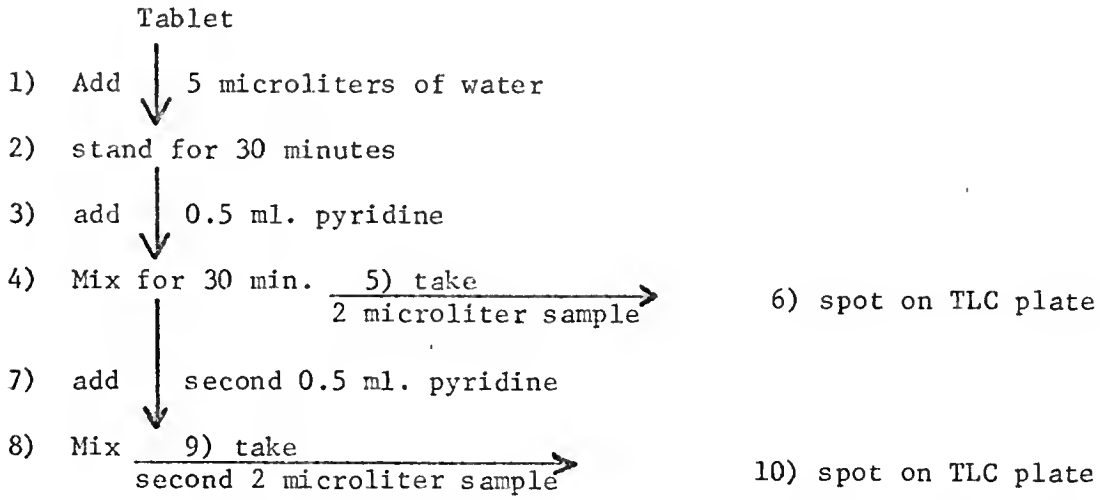
The reason for choosing this scheme over one that would involve attachment of the m-dinitrobenzene to the opened lactone ring is that the added conjugation would not lower the energy of the system enough to make it visible. An electron transfer, however, followed by a complexation step would lower the energy enough to explain the large change in absorbance wave length.

A standard curve was produced using five different concentrations of digoxin in alcoholic solution. They were treated according to the U.S.P. method and the results appear in Figure I.

Thin Layer Chromatography

Thin layer chromatography was performed on Adsorbosil-5-Prekotes supplied by Applied Science, College Park, Pennsylvania. The solvents were supplied by Matheson, Coleman and Bell, Atlanta, Georgia. Various grades of solvents were used. Pyridine, cyclohexane, acetone and acetic anhydride were Spectra grade, while the ethanol was U.S.P. 95 per cent. All other solvents were Technical grade. Pure powdered digoxin was purchased from K & K Labs, Plainview, N.Y., Lot #15614, as were the pure powdered samples of the aglycons. All powdered samples were tested for purity using thin layer chromatography.

The samples were assayed quantitatively by the following procedure: A digoxin tablet was removed from the desiccator and placed in a 5 mm. test tube. Fifty microliters of distilled water were then added to the test tube and allowed to stand for 30 minutes. At this point 0.5 ml. of pyridine was added, and the mixture agitated for 30 minutes at 5 minute intervals. A 2 microliter aliquot was removed from the clear solution and spotted on a plate. An additional 0.5 ml. aliquot of pyridine was then added, mixed, and a second 2 microliter portion was spotted on the same plate. A 2 microliter aliquot from a known standard solution was also spotted. Its concentration was estimated to be between that of the two unknown aliquots. Step by step the assay procedure is as follows:



The plate was then developed in glass unlined tanks using a mixture of cyclohexane, acetone and acetic acid (49-49-2). The plate was removed after the solvent front had migrated the full distance of the plate and dried before visualization, employing the spray reagent.

A spray consisting of a mixture of a 3 per cent aqueous solution of chloramine T and a 25 per cent alcoholic solution of trichloroacetic acid was prepared. The two solutions, in a ratio of 1:4, were mixed

just prior to use. After spraying, the plate was heated in an oven at 110° for seven minutes and a blue spot was observed under UV light (385 mμ). The chemical reaction which could possibly explain the generation of the fluorescence is the oxidation of the lactone ring to a diketone which when protonated will fluoresce.

The color development for both the U.S.P. assay and the thin layer visualization are non-specific reactions for any compound which has a lactone ring. The specificity of the thin layer procedure arises from the separation step preceding the quantification.

During the course of the stability studies, single tablets were taken at random from a group of tablets being exposed to each of the nine sets of conditions. The assay described in the discussion of thin layer chromatography was then performed and the results tabulated for analysis. The data was analyzed for linear and exponential fit, using two programs currently used by the University of Florida Computer Center. Further statistical calculations were carried out on a standard desk calculator supplied by Monroe. An analysis of variance, according to a 2x3x3 factorial design, was performed on the linear regression coefficients. This analysis demonstrates the effect of temperature, humidity and method of manufacture of the digoxin tablets.

RESULTS AND DISCUSSION

An exhaustive attempt to reproduce the gas chromatographic assay proposed by Wilson was made. It was possible only to obtain good results for the pure genin. The glycosides showed no detectable peak under any of the conditions used. This may have been due to the low temperatures at which digoxin has been reported to degrade, as well as the high vapor pressure produced by the high molecular weight compound. At low oven temperatures, the compound moves so slowly that it just gives rise to base line drift and noise, while at high temperatures, it fragments and comes off as small pieces which has the same effect as base line drift.

It was possible, however, to identify the pure genin (aglycone). To quantify the amount of each of the glycosides (digoxin, monodigitoxose, digoxigenin and bis-digitoxose digoxigenin) would require a hydrolysis step following either thin layer or column chromatography. The pure genin could then have been assayed and the amount of parent compound calculated. This method would require many time consuming manipulative steps and probably introduce unnecessary errors. As a result, it was decided to rely on quantitative thin layer chromatography as the main method of assay.

The twenty tablets, assayed by the U.S.P. method before the start of the stability study, showed an average content of 0.25 mg. of digoxin per tablet. When the assay was repeated at the end of the study on 20 tablets taken from each of the nine conditions to which they were

subjected they again showed an average content of 0.25 mg. per tablet. The fact that no change in total glycoside content was evident is a result of the type of assay used. Since the assay depends on a reaction that occurs at the lactone ring, the presence of one, two or three sugars on the other end of the steroid has no effect. It is possible, therefore, to assay a group of tablets which have been completely hydrolyzed to the pure genin or partly degraded through removal of one or two digitoxose sugars and still obtain results in terms of the parent compound. For this reason the U.S.P. assay could not be used as a means for determination of the amount of degradation which occurred in the tablet. It is true that the U.S.P. assay could be adjusted to evaluate a single tablet, but only in terms of total glycoside content, not in terms of the four possible compounds of interest in this study.

Thin Layer Chromatography

Figure 2 shows a plot of the logarithm of the weight of digoxin spotted versus the square root of the area of the developed spot for a series of solutions. Eleven different solutions were prepared by dilution of a stock solution and randomly spotted in duplicate (two microliter spots on six different plates). No attempt was made to prepare even numbers of replicates as the graph was not intended to be used as a standard curve but only as a test for linearity. The scatter about the line can be partially attributed to a plate to plate variation. Each silica gel plate used in the thin layer chromatography assay cannot be prepared in exactly the same manner, which affects the area covered by a given weight of digoxin. Table II shows a comparison of six plates chosen at random. An analysis of variation was done using the plate as the treatment. The large F shows that there is a

statistically significant effect of the plate on the size of a spot from a given amount of drug. Consequently, all weights of unknown samples were calculated mathematically by an area comparison between the unknown and a standard solution of digoxin spotted on the same plate. This procedure eliminates any plate to plate variation which would have otherwise occurred.

The weight of the unknown was calculated using the following formula: $\text{Log } W = \text{Log } W_s + [(\sqrt{A} - \sqrt{A_s})/(\sqrt{A} - \sqrt{A_d})](\log d)$

Where W = weight of unknown sample

W_s = weight of standard sample

A = spot area of the unknown

A_s = spot area of the standard

A_d = spot area of the diluted unknown sample

d = dilution factor

A straight line relationship between log W and $\sqrt{A}$ has been demonstrated to be true using a series of standard solutions (Figure 2).

Assuming coordinate $(\sqrt{A}, \log W)$, $(\sqrt{A_s}, \log W_s)$, and $(\sqrt{A_d}, \log W/d)$ to be three points on the line, the slope can be calculated as $(\log W - \text{Log } W_s)/(\sqrt{A} - \sqrt{A_s})$ or $(\text{Log } W - \text{Log } W/d)/(\sqrt{A} - \sqrt{A_d})$.

Equating the two:

$$\frac{\text{Log } W - \text{Log } W_s}{\sqrt{A} - \sqrt{A_s}} = \frac{\text{Log } W - \text{Log } W/d}{\sqrt{A} - \sqrt{A_d}}$$

Combining the terms:

$$\frac{\text{Log } W - \text{Log } W_s}{\sqrt{A} - \sqrt{A_s}} = \frac{\text{Log } d}{\sqrt{A} - \sqrt{A_d}}$$

Rearranging:

$$\text{Log } W - \text{Log } W_s = [(\sqrt{A} - \sqrt{A_s})/(\sqrt{A} - \sqrt{A_d})](\log d)$$

Finally:

$$\text{Log } W = \text{Log } W_s + [(\sqrt{A} - \sqrt{A_s})/(\sqrt{A} - \sqrt{A_d})](\log d)$$

The solvent system was chosen on the basis of the difference in Rf values between digoxin and its genins. This would allow easy identification of simple hydrolysis products since these would show up higher on the plate than digoxin. The pure sugar digitoxose was found to appear well below the digoxin spot. Other components of the tablet such as talc, starch and stearic acid were found to remain behind at the original point of spotting and were not considered as interfering with the assay. Table III shows the Rf values for the various solvent systems. Based on these findings, a combination of cyclohexane-acetone-acetic acid (49-49-2) was chosen as the solvent system with a one-half hour drying time between two successive runs. This procedure gave maximum separation.

The degradation studies were carried out by assaying a tablet taken from each desiccator at seven-day intervals. The results obtained along with their analysis of variance and test for significance are shown in Tables IV-XXI. The degradation data was analyzed by the use of two programs already in the computer. These programs treated the data first as a linear model and then as an exponential one. A comparison of the residual mean squares of the two fits shows that the linear model values are all smaller than those of the exponential one indicating that the latter is not better and probably worse than the linear fit. On this basis the simpler model was chosen. The comparison of the mean squares is shown in Table XXII.

It is not to be inferred from the above discussion that in some instances a non-linear component does not exist. It is not, however,

exponential in nature. The choice of the linear model was based, therefore, on the fact that it would provide a reasonably close approximation of the degradation kinetics at play. The coefficient of regression was chosen as the statistical parameter for the evaluation of the temperature, humidity, and manufacturer effects.

The various coefficients of regression were then treated using 3x3x2 factorial design shown in Table XXIII. An analysis of variance was performed and the results appear in Table XXIV. The data clearly indicates a significant effect due to temperature and humidity and a slight effect due to manufacturing differences between tablets.

The results of three control routine tests performed on the manufactured tablets are shown in Table XXV. They indicate the hardness, weight and tablet to tablet weight variation and the disintegration times of a sample of each of the groups of tablets used in the stability studies. Note that the Purepac brand is harder and disintegrates slower than the Burroughs Wellcome brand, as well as the weight, on the average, being ten milligrams more. There is also more variation in tablet weight and hardness in the Purepac brand than with the Burroughs Wellcome brand as indicated by the large standard deviation for both of these parameters. The results may be explained in a number of ways. It is most probable that the Purepac Company intended to produce a harder tablet so that fewer tablets would be lost due to breakage during manufacture, packaging and storage. This made it necessary to add a stronger binder or more binder in the base formulation and subjected the tablet to a possibly greater pressure during the compression cycle of the tabletting operation. Purepac has also succeeded in obtaining

a good disintegration rate which is probably due to a disintegrant in the formulation. These two changes possibly explain the added ten milligrams of weight of the average tablet. Variation in granulation particle size and improper classification of the granulation before compression would lead to a larger weight variation from tablet to tablet. These slight changes in total formulation would show up later in the stability studies as a significant effect on overall resistance to hydrolysis.

As can be seen in Table IV and Table XIII, the F ratio is low. This signifies that the coefficient of regression for these two sets of conditions cannot be said, with any confidence, to vary from zero. On the other hand, a comparison of the F ratio for more strenuous conditions of temperature and humidity, as seen in Tables XII and XXI, shows that a definite slope is apparent. The first fact is a result of the high tablet to tablet variation as well as the very slight rate of degradation of digoxin. When all of the regression coefficients are considered together, ignoring their relative F ratios, a trend is apparent that justifies the overall analysis of variance that was performed.

In Table XXIV the effect of humidity showed the largest F ratio which would indicate that it is the single most important effect. From this fact, it could be deduced that in the absence of moisture, no reaction would occur and that the primary reaction being considered here must involve water in the transition state. The large positive F for temperature indicates an increase in reaction rate with an increase in temperature as would be expected in any reaction which must overcome an energy peak as postulated by Arrhenius. However, when an attempt

was made to fit the data for the coefficients of regression on a standard Arrhenius plot, it appears to hold only at the lowest humidity. As the humidity is increased, the linearity of the plot is lost and a curve results. The high F ratio for the cross term, humidity x temperature, gives an indication that this would occur. This might be accounted for by the effect of humidity on the apparent energy of activation. The data indicates that the higher the humidity, the higher the apparent energy of activation (Figure 3). This fact might at first glance seem opposite to what one would expect, since the increase in humidity should in all probability increase the rate but not change the energy of activation. If indeed the same energy state or transition state is in operation in all cases, the increase in humidity while affecting the rate should not in any way affect the slope of the Arrhenius plot. As indicated, however, the results are not in accord with this line of reasoning. The most obvious explanation appears to be that the parameters actually being measured are two different energy pathways and that the rate determining step is shifting from the one dependent on the transfer of water through the tablet to the one dependent on the reaction time of digoxin with water. One could look at the overall reaction as involving two steps. The first is the transfer of water from the atmosphere to the site of hydrolysis and the second being the actual hydrolysis itself due to moisture present. This is to say that at low humidity the adsorption and diffusion effects are regulating factors, while at higher humidities most sites for reaction are saturated with water, and the above factors no longer predominate. This can be visualized in the following scheme:

$$\text{Water} \xrightarrow{k_1} \text{water \& digoxin} \xrightarrow{k_2} \text{genin \& digitoxose}$$

in which k_1 is a constant of diffusion and k_2 is the rate constant for hydrolysis. Accordingly, if the slope of the Arrhenius plot is examined in Figure 3 for the 18.8 per cent relative humidity conditions, an energy of activation is obtained of approximately 4 kilocalories/mole while the shift in slope for the 80.5 per cent relative humidity resulted in a calculated energy of activation of 13 kilocalories/mole.

Any tablet, regardless of how well formulated and manufactured, cannot be as uniform in solid form concentration as in solution. Tablets are made by compressing granules of a drug together with inactive ingredients, while solutions allow for dissolution of the drug on the molecular level. Crystal size of an active drug will affect the amount of surface exposed to the moisture and thereby its chances for hydrolysis. The various mixing and other processes involved in granulation and tablet preparation are bound to affect the uniformity of drug concentration across the tablet. A granulation, whether it is prepared by the slugging process or by the wet process, will contain fine and coarse particles which are liable to stratify. Furthermore, migration in a tablet granulation can occur through routine handling and if the active ingredient happens to be concentrated in one particular area, then, upon compression, irregularities in the amount of active ingredient will occur. These factors may be largely responsible for tablet to tablet variation which causes each tablet to contain a different initial amount of drug. It is, therefore, difficult or impossible to establish a zero time level. Each tablet acts as its own reaction vessel and the nature of that vessel changes from tablet to tablet. Porosity, hardness and total weight thus contribute to creating a

different environment for reaction. Also, the change in the amount of various additives, such as starch, can change the rate at which the humidity in the atmosphere can migrate through the tablet to the site of hydrolysis.

Most manufacturers are conscious of the problems involved with possible concentration variation among tablets. The fact remains that even with the greatest of manufacturing care, no two tablets can be exactly alike. This fact is reflected in the large scatter about the regression in each of the sets of data for the various conditions. It also shows up in Table XXIV as a significant F ratio for manufacturer effect.

To analyze the results in terms of solution kinetics would oversimplify the situation. In solution kinetics, factors such as pH, concentration, temperature and time can all be measured with a great degree of accuracy. In a tablet there is a given amount of the drug per total weight of tablet but this does not really qualify as an absolute concentration. The active ingredient is not dispersed uniformly throughout the tablet as it would be in a solution. It is in pockets of pure crystal and reacts as a pure crystal. If the hydrolysis is assumed to require dissolution on the drug into a multimolecular layer of water adsorbed on the surface of the crystal, then the solubility of the drug will affect the overall rate and the scheme must be expanded to include solution rate.

In solution kinetics, there can be an optimum pH for stability of the drug. In solid state systems, the pH or hydrogen ion concentration at the site of reaction is fixed by the solubility of the drug and the

additives adjacent to it. Therefore, in solid state kinetics, the pH at the site of reaction can vary from tablet to tablet and even within one tablet.

The humidity can be considered to be directly proportional to the amount of water at the site of reaction, only as long as diffusion through the tablet is a rate limiting step. Thus at low humidities, the less porous the tablet, the slower the rate of reaction. The type of "inert" ingredient may either slow or speed the passage of water through the tablet. If it is hydrophobic in nature, it can retard degradation, whereas if it is hydrophilic, it might increase the rate of degradation.

As a result of this study, there is a need for continuous monitoring on a tablet to tablet basis of lots from all the manufacturers of potent pharmaceuticals. It is not possible to make the normal prediction of stability based on the Arrhenius relationship which only applies to solution kinetics. The need now exists for the development of theory and methodology that can adequately describe the type of reaction which occurs in most tablets on the market, so as to make valid long range stability predictions based on current data.

APPENDIX

A. Tables

TABLE I

RELATIVE HUMIDITIES EMPLOYED

Relative Humidity	Density of Solution	W/W % Sulfuric Acid
80.5%	1.20	28%
47.2%	1.35	45%
18.8%	1.50	60%

TABLE II

TLC PLATE TO PLATE VARIATION
(USING 0.799 MICROGRAMS OF DIGOXIN)

	1	2	3	4	5	6	Total
$\sqrt{A}$	0.1457	0.1581	0.1541	0.1658	0.1904	0.2179	
	0.1412	0.1732	0.1541	0.1936	0.2208	0.1969	
	0.1500	0.1868	0.1616	0.1694	0.1935	0.1837	
	0.1500	0.1732	0.1581	0.1541	0.2000	0.1968	
	0.1541	0.1837	0.1581	0.1803	0.2150	0.1801	
	0.1411	0.1658	0.1498	0.1904	0.2398	0.1801	
n	6	6	6	6	6	6	36
Sum of X	0.8821	1.0408	0.9319	1.0536	1.2595	1.1555	6.3213
Sum of X^2	0.12982	0.18112	0.14479	0.18617	0.26617	0.22359	1.13166
$\frac{(\text{Sum of } X)^2}{6}$	0.1297	0.1805	0.1447	0.1850	0.2644	0.2225	
$\bar{X}$	0.1470	0.1735	0.1553	0.1750	0.2099	0.1926	

Analysis of Variance

Source	df	Sum of Squares	Mean Square	F Ratio
Among plates	5	0.01684	0.00336	21.00***
Within plates	30	0.00486	0.00016	
Total	35	0.02170		

*** Significant at the 0.1% level ($P<.001$)

TABLE III

THIN LAYER CHROMATOGRAPHY Rf VALUES
FOR VARIOUS SOLVENT SYSTEMS

Solvent System	Rf Values			
	D	D_1	D_2	Digoxin
Ethyl Acetate	0.3708	0.3708	0.1685	0.1348
Ethyl Acetate-Pyridine-Water (50-10-40)	0.8108	0.8784	0.8243	0.8108
Cyclohaxane-Acetone-Acetic Acid (49-49-2)	0.3537	0.3049	0.2805	0.2561
Same as above but run twice with drying between	0.7381	0.6548	0.5595	0.5000
Ethyl Acetate-Methanol-Water (90-5-5)	0.7561	0.7560	0.6585	0.6219
Benzene-Ether (70-30)	0	0	0	0
Benzene-Ether-Ethanol (70-30-20)	0.041	0.041	0.0137	0.0137
Benzene-Ethanol (82-18)	0.2812	0.2187	0.2031	0.1875
Benzene-Ethanol (70-30)	0.9354	0.8871	0.7903	0.7742
Benzene-Ethanol (82-23)	0.6571	0.6428	0.6143	0.6000
Ethyl Acetate-Methanol-Water (16-1-1)	0.6969	0.6666	0.5909	0.5606
Ethyl Acetate-Methanol (80-10)	0.8286	0.8714	0.8570	0.8429

Key: D = digoxigenin; D_1 = digoxigenin mono-digitoxose; D_2 = digoxigenin bis-digitoxose.

TABLE IV

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
23° AND 18.8% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
39	0.995	91	1.580
50	1.240	98	1.240
56	1.110	105	1.240
70	1.950	114	0.951
77	1.160	120	0.651
84	0.996		

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.076	0.076	0.627
Residual	9	1.092	0.121	

Variables in Equation

Coefficient	-0.00328
Standard Error	0.00415
F to Remove	0.6271

TABLE V

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
23° AND 47.2% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
38	1.020	84	1.150
50	0.992	91	0.961
56	1.310	98	0.618
63	1.200	105	1.020
70	1.540	114	0.595
77	1.260	120	0.612

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.379	0.379	6.375
Residual	10	0.595	0.059	

Variables in Equation

Coefficient	-0.00712
Standard Error	0.00282
F to Remove	6.3753

TABLE VI

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
23° AND 80.5% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
39	1.080	84	1.110
50	1.550	91	0.802
56	1.190	98	1.040
63	1.600	105	0.803
70	1.590	114	1.090
77	0.976	120	0.581

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.501	0.501	7.558
Residual	10	0.662	0.066	

Variables in Equation

Coefficient	-0.00823
Standard Error	0.00299
F to Remove	7.5584

TABLE VII

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
40° AND 18.8% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
38	0.745	84	1.110
45	1.190	91	0.966
50	0.804	98	0.854
56	1.310	105	0.899
63	1.100	114	0.502
70	1.030	120	0.434
77	0.894		

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.250	0.250	5.304
Residual	11	0.518	0.047	

Variables in Equation

Coefficient	-0.00537
Standard Error	0.00233
F to Remove	5.3040

TABLE VIII

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
40° AND 47.2% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
38	1.350	84	1.060
45	1.024	91	1.028
50	1.590	98	1.600
56	1.240	105	0.317
63	1.410	114	0.634
70	1.110	120	0.725
77	0.990		

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.630	0.630	6.735
Residual	11	1.030	0.094	

Variables in Equation

Coefficient	-0.00854
Standard Error	0.00329
F to Remove	6.7352

TABLE IX

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
40° AND 80.5% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
38	1.508	84	1.400
45	0.988	91	0.877
50	1.150	98	0.854
56	1.360	105	0.888
63	1.180	114	0.498
70	1.050	120	0.513
77	0.900		

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.716	0.716	18.121
Residual	11	0.435	0.040	

Variables in Equation

Coefficient	-0.00911
Standard Error	0.00214
F to Remove	18.1205

TABLE X

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
50° AND 18.8% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
43	0.707	84	1.050
50	0.799	98	1.400
56	1.260	105	0.274
63	1.690	114	0.583
70	1.590	120	0.632
77	1.050		

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.314	0.314	1.660
Residual	9	1.705	0.189	

Variables in Equation

Coefficient	-0.00672
Standard Error	0.00522
F to Remove	1.6601

TABLE XI

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
50° AND 47.2% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
43	0.876	84	1.070
50	1.120	91	1.040
56	0.679	98	0.863
63	1.910	105	0.294
70	1.970	114	0.683
77	1.220	120	0.672

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.522	0.522	2.434
Residual	10	2.146	0.215	

Variables in Equation

Coefficient	-0.00859
Standard Error	0.00551
F to Remove	2.4339

TABLE XII

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY PUREPAC
50° AND 80.5% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
43	1.360	84	1.420
50	1.320	91	0.515
56	1.810	98	0.208
63	1.800	105	0.114
70	1.590	114	0.961
77	1.430	120	0.667

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	2.000	2.000	10.647
Residual	10	1.878	0.188	

Variables in Equation

Coefficient	-0.01681
Standard Error	0.00515
F to Remove	10.6472

TABLE XIII

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
23° AND 18.8% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
38	1.110	84	1.580
45	0.749	91	0.887
50	0.736	98	1.860
56	1.590	105	0.992
63	1.470	114	0.698
77	1.420	120	0.227

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.135	0.135	0.567
Residual	10	2.387	0.239	

Variables in Equation

Coefficient	-0.00397
Standard Error	0.00528
F to Remove	0.5669

TABLE XIV

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
23° AND 47.2% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
38	1.024	84	1.200
45	0.905	91	0.627
50	0.834	98	1.500
56	1.330	105	0.056
63	1.050	114	0.745
77	1.270	120	0.789

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.172	0.172	1.190
Residual	10	1.442	0.144	

Variables in Equation

Coefficient	-0.00447
Standard Error	0.00410
F to Remove	1.1896

TABLE XV

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
23° AND 80.5% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
38	1.094	84	1.200
45	0.798	91	0.574
50	0.438	98	1.390
56	0.854	105	0.039
63	0.799	114	0.799
77	1.140	120	0.124

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.193	0.193	1.114
Residual	10	1.728	0.173	

Variables in Equation

Coefficient	-0.00474
Standard Error	0.00449
F to Remove	1.1142

TABLE XVI

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
40° AND 18.8% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
43	1.260	84	1.210
50	1.470	91	0.711
56	1.230	98	1.410
63	0.488	114	0.999
70	1.390	120	0.784
77	0.518		

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.105	0.105	0.785
Residual	9	1.205	0.134	

Variables in Equation

Coefficient	-0.00404
Standard Error	0.00456
F to Remove	0.7851

TABLE XVII

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
40° AND 47.2% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
43	1.140	84	1.280
50	1.230	91	1.170
56	1.380	98	1.830
63	1.770	114	0.799
70	1.420	120	0.481

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.320	0.320	2.233
Residual	8	1.147	0.143	

Variables in Equation

Coefficient	-0.00705
Standard Error	0.00472
F to Remove	2.2331

TABLE XVIII

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
40° AND 80.5% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
43	1.020	84	1.100
50	1.330	91	0.849
56	1.600	98	0.950
63	1.250	114	0.819
70	1.140	120	0.677

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.406	0.406	12.298
Residual	8	0.264	0.033	

Variables in Equation

Coefficient	-0.00794
Standard Error	0.00226
F to Remove	12.2984

TABLE XIX

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME 50° AND 18.8% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
50	1.180	91	0.914
56	0.027	98	1.200
63	1.130	105	0.809
70	1.650	114	0.799
84	1.090	120	0.196

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.128	0.128	0.520
Residual	8	1.968	0.246	

Variables in Equation

Coefficient	-0.00484
Standard Error	0.00672
F to Remove	0.5195

TABLE XX

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
50° and 47.2% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
50	1.380	91	0.639
56	0.799	98	1.640
63	1.710	105	1.190
70	1.460	114	0.509
77	0.925	120	0.696
84	0.885		

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	0.346	0.346	2.196
Residual	9	1.419	0.158	

Variables in Equation

Coefficient	-0.00793
Standard Error	0.00535
F to Remove	2.1960

TABLE XXI

THE DEGRADATION OF DIGOXIN TABLETS SUPPLIED BY BURROUGHS WELLCOME
50° AND 80.5% RELATIVE HUMIDITY

Time in Days	Micrograms of Digoxin	Time in Days	Micrograms of Digoxin
50	1.270	91	0.847
56	1.130	98	1.040
63	1.570	105	0.545
70	1.600	114	0.607
84	1.110	120	0.035

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Regression	1	1.471	1.471	18.760
Residual	8	0.627	0.078	

Variables in Equation

Coefficient	-0.01643
Standard Error	0.00379
F to Remove	18.7600

TABLE XXII

A COMPARISON OF RESIDUAL MEAN SQUARES
FOR LINEAR AND EXPONENTIAL FITS

	Linear	Exp.
1	0.121	0.122
2	0.059	0.062
3	0.066	0.070
4	0.047	0.050
5	0.094	0.097
6	0.040	0.043
7	0.189	0.194
8	0.215	0.223
9	0.188	0.193
10	0.239	0.241
11	0.144	0.146
12	0.173	0.175
13	0.134	0.133
14	0.143	0.149
15	0.033	0.036
16	0.246	0.248
17	0.158	0.160
18	0.078	0.114

TABLE XXIII

3X3X2 FACTORIAL DESIGN OF COEFFICIENTS OF REGRESSION

		H_1	H_2	H_3	Total
T_1	M_1	3.28	7.12	8.23	18.63
	M_2	3.97	4.47	4.74	13.18
	Total	7.25	11.59	12.93	31.81
T_2	M_1	5.37	8.54	9.11	23.02
	M_2	4.04	7.05	7.94	19.03
	Total	9.41	15.59	17.05	42.05
T_3	M_1	6.72	8.59	16.81	32.12
	M_2	4.84	7.93	16.43	29.20
	Total	28.22	43.70	63.26	135.18

Key: M_1 = Purepac; M_2 = Burroughs Wellcome

H_1 = 18.8% relative humidity; H_2 = 47.2% relative humidity;

H_3 = 80.5% relative humidity

T_1 = 23°; T_2 = 40°; T_3 = 50°

For ease of analysis all values were multiplied by -100.

TABLE XXIV

ANALYSIS OF VARIANCE OF COEFFICIENTS OF REGRESSION

Source	Df	Sum of Squares	Mean Square	F Ratio
Humidity	2	102.7792	51.3896	41.90**
Temperature	2	74.8350	37.4175	30.51**
Manufacturer	1	8.4872	8.4872	6.86#
Humidity X Temperature	4	51.6099	12.9025	10.52*
Humidity X Manufacturer	2	0.6448	0.3224	0.26
Temperature X Manufacturer	2	0.5377	0.2688	0.22
Error	4	4.9058	1.2264	---
Total	17	243.7996	---	---

** Significant at the 1% level (P<.01)

* Significant at the 5% level (P<.05)

Significant at the 10% level (P<.10)

TABLE XXV

A COMPARISON OF WEIGHT VARIATION, HARDNESS, AND DISINTEGRATION RATE OF TABLETS

Purepac		Burroughs Wellcome	
Tablet weight in milligrams		Tablet weight in milligrams	
135.8	140.2	122.7	124.4
136.8	137.0	126.8	125.5
134.5	134.8	124.8	129.8
135.0	134.7	128.4	124.6
138.3	136.0	127.5	126.5
131.3	137.3	125.8	123.5
132.6	134.1	125.7	125.5
131.0	136.6	126.2	124.9
134.4	135.7	125.6	126.7
136.4	137.3	126.6	124.7
Sum of X	2709.8	2516.3	
Sum of X^2	367,244.2	316,638.73	
$\bar{X}$	135.49	125.82	
s	2.217	1.629	
Hardness in Kilograms		Hardness in Kilograms	
4.0	3.0	1.5	1.5
3.5	3.0	1.5	1.5
5.0		2.0	
Sum of X	18.5	8	
Sum of X^2	71.25	13.0	
X	3.7	1.6	
s	0.836	0.224	
Disintegration rate in seconds		Disintegration rate in seconds	
21	22	16	18
23	23	17	16
22		17	
Sum of X	111	84	
Sum of X^2	2467	1414	
$\bar{X}$	22.2	16.8	
s	0.836	0.836	

B. Figures

FIGURE I

STANDARD CURVE FOR U.S.P. ASSAY

A = Absorbance measured on the Fisher Colorimeter

C = Concentration of Digoxin in mg/ml.

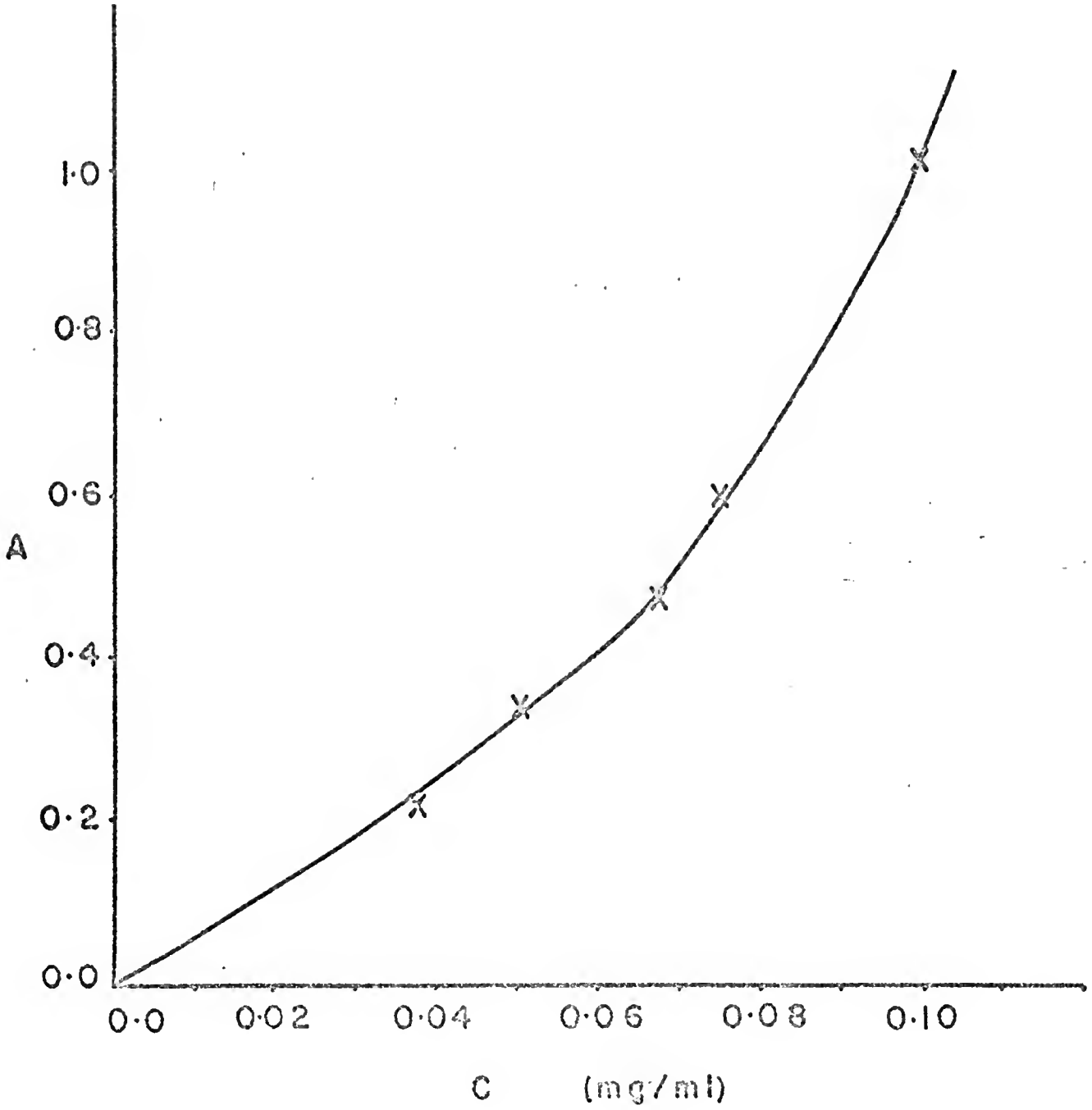

1·0
0·8
0·6
A
0·4
0·2
0·0
0·0
0·02
0·04
0·06
0·08
0·10
C (mg/ml)

FIGURE II

STANDARD CURVE FOR THIN LAYER CHROMATOGRAPHY

The square root of the area of the spot is plotted versus the log of the weight of Digoxin in µg. spotted.

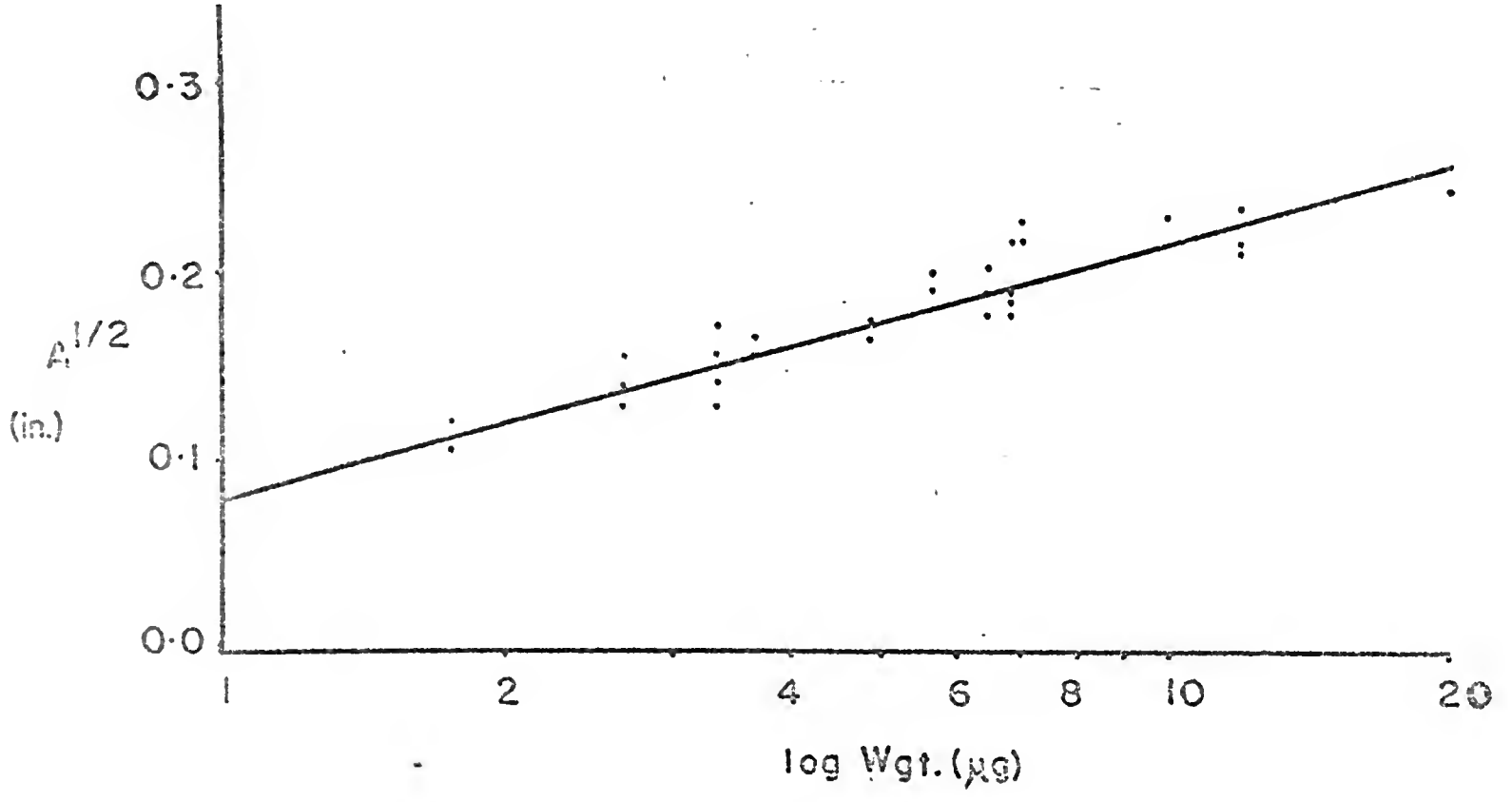

0·3
0·2
0·1
0·0
A^1/2
(in.)
1
2
4
6
8
10
20
log Wgt. (µg)

FIGURE III

ARRHENIUS PLOT OF REGRESSION COEFFICIENTS

The linear regression coefficients are plotted versus the inverse of the temperature in degrees Kelvin.

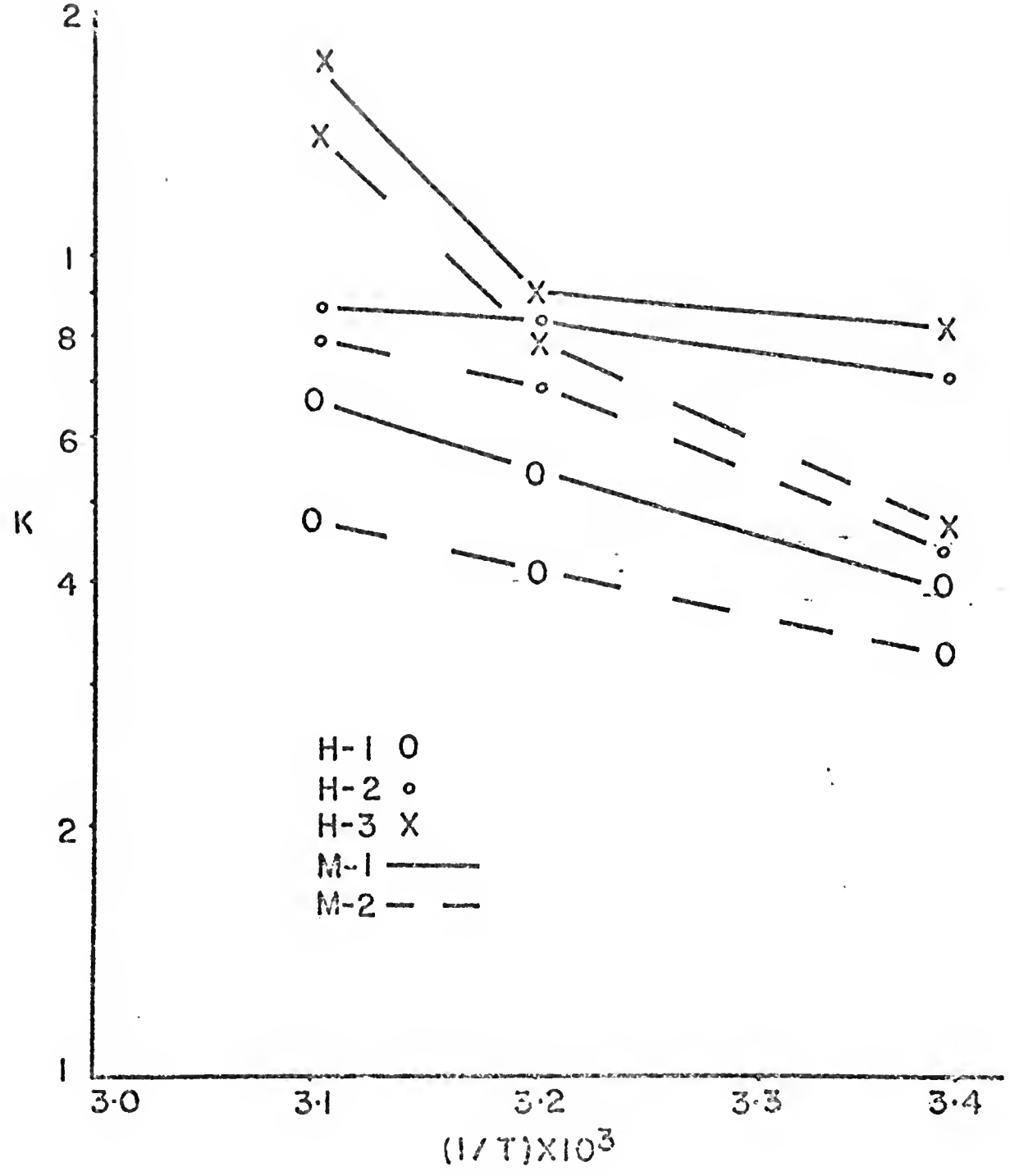
Key: M-1 = Purepac

M-2 = Burroughs Wellcome

H-1 = 18.8% Relative Humidity

H-2 = 47.2% Relative Humidity

H-3 = 80.5% Relative Humidity


2
1
8
6
K
4
2
1
3·0
3·1
3·2
3·3
3·4
(1/T)X10³
H-1 O
H-2 o
H-3 X
M-1
M-2

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BIOGRAPHICAL SKETCH

Arthur H. Kibbe was born February 10, 1943 at Staten Island, New York. He enrolled in the College of Pharmacy, Columbia University, where he received the degree of Bachelor of Science in Pharmacy in June 1966.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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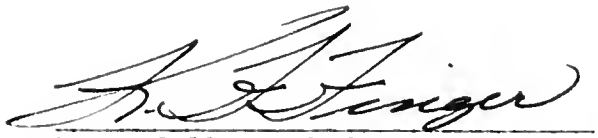
March, 1973



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